A SYNOPSIS ON

IN VITRO PLANT GROWTH-PROMOTION (PGP) ACTIVITIES AND MOLECULAR CHARACTERIZATION OF ENDOPHYTIC ROOT-NODULE BACTERIA ISOLATED FROM CULTIVATED LEGUMES (*CICER ARIETINUM* L. AND *VIGNA ACONITIFOLIA* L.) OF SEMI-ARID AND ARID REGIONS OF RAJASTHAN, INDIA

SUBMITTED TO

DEPARTMENT OF SCIENCE
FACULTY OF ARTS SCIENCE AND COMMERCE (FASC)
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Under Supervision of:
Dr. D. K. Choudhary

Submitted by:
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M. Sc. (Biotechnology)

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INTRODUCTION TO PROBLEM AND OBJECTIVES

1. INTRODUCTION

The changes in the economic conditions of agricultural production, overproduction in the traditional agricultural communities, and the deriving marketing difficulties all urged both researchers and agricultural producers to look for plant species which could be produced in an economically viable way, which expand biodiversity, and which can be fitted into the applied (sustainable) producing systems. The legumes are extra-ordinarily diverse in every way and are important in different agricultural and natural environment. They are used to restore or increase the soil fertility of degraded soil in intercropping systems, as green manure and to produce medicinal and commercial by-products. As a hallmark trait legume develop root nodules and fix atmospheric nitrogen in symbiosis with compatible bacteria. These compatible bacteria are soil bacteria (rhizobacteria) and promote growth of plants directly or indirectly, therefore referred as Plant Growth Promoting Rhizobacteria (PGPR). In fragile ecosystem, the PGPR play important role, however the symbiotic relationship of PGPR and legumes is influenced by various factors. The PGPR encounter various kinds of environmental stresses in rhizosphere i.e. desertification, salinity, temperature extremes, unfavourable soil pH, nutrient deficiency and other kind of stresses. These stresses adversely affect the growth of PGPR, initial steps of symbiosis and the capability of nitrogen fixation. A fully functional symbiosis between a host and PGPR requires

successful survival of bacteria even under adverse environmental conditions. A competitive and persistent rhizobacterial or PGPR strain associated with crops is not expected to express its full potential for nitrogen fixation as the limiting factors impose limitations on the vigour of the host legume and potentiality of symbiotic relationship. The leguminous crops commonly grown in arid and semi-arid regions are subjected to severe environmental conditions. There are strong possibilities that these crop plants of arid and semi-arid regions may harbor PGPR, which may express full potential under the existing adverse environmental conditions. Inoculation of these efficient strains or stress tolerant strains of rhizobia may enhance the nodulation and nitrogen fixation ability of other plant species under stressed conditions.

Most of parts of Rajasthan state (Approximately 60%) falls within the desert region (34°35'N to 30°10'N latitude and 69°31'E to76°55'E longitude) and covers the largest area (61%) of hot arid regions of the Indian Desert (DST, 1994). Several leguminous plants find their habitats in hot arid regions and the microbial diversity associated with these plants is one of the most useful resources for bioprospecting. In Rajasthan the cultivated leguminous crops include *Pisum sativum*, *Vigna* species (*V. aconitifolia*, *V. mungo*, *V. Radiata*, *V.unguiculata* etc.), *Cicer aeritinum*, *Cymopsis tetragonoloba*, *Trigonella foenumgraecum*, *Cajanus cajan*, *Lens culinaris*, *Vicia faba*, *Lablab purpureus*, *Phaseolus lunatus*, *Canovalia ensiforis*, *Arachis hypogea* etc. Beside cultivated crops, a number of native legumes also grow in this region. These plants are important resources include native and cultivated crops. The crops grown in hostile environment are important plant resources. The natural rhizobacterial associates of these crops might exhibit higher tolerance to prevailing adverse environmental conditions. Therefore, these legumes (with their PGPR associates) are the one of alternatives to costly and pollutive mineral nitrogen fertilizers used in agriculture. In these legumes growing under arid and semi-arid environment, nitrogen fixation studies have been largely ignored. Therefore these plants are important for studies as there is strong possibility that these plants may harbor those rhizobia or soil bacteria, which might play important role for increasing productivity of other cultivated legumes and for large-scale multiplication of other crops/plants in arid regions of Indian Thar Desert. Selection of

effective, efficient and compatible stress tolerant rhizobial strains could help in ecological rehabilitation of degraded soils and to increase soil fertility thereby improving the growth of plants of this region.

Considering these constrains, the proposed project work is designed to isolate and phylogenetically characterize the PGPR from crops commonly grown in arid and semi-arid regions of Rajasthan for their multiple plant growth promoting (PGP) activities. Also the application of non-conventional approaches may further provide information regarding nodulation and plant microbe interactions. More so, the study will contribute to microbial biodiversity of rhizobia or soil bacteria with legumes in hostile desert ecosystem.

INTRODUCTION TO PLANT SPECIES SELECTED FOR INVESTIGATION

A. Chickpea (*Cicer arietinum* L.):

Chickpea [*Cicer arietinum* (L.)] is a self-pollinated diploid (2n= 16) grain leguminous crop, taxonomically classified to separate tribe Cicereae in subfamily Papilionaceae/Faboideae of family Fabaceae (Kupicha, 1981). Chickpea is an important grain legume in West Asia, Northeast Africa, South Europe, South-Central America and Australia (FAOSTAT Report, 2007). Chickpea is called chana (Hindi), and gram or Bengal gram (English) and originated in South-Eastern Turkey (Ladizinsky, 1975). Cultivated chickpea (*Cicer arietinum* L.) is the second most important cool season pulse crop in the world (Singh, 1997). Chickpea is the world's third most important pulse crop and and India accounts for 75 % of the world's chickpea production. Chickpea is good as a source of carbohydrate (48.2-67.6 %), protein (12.4-31.5 %), fat (6%) and nutritionally important minerals. Among the legumes chickpea is the best hypocholesteremic agent, followed by black gram and green gram.

B. Fenugreek (*Trigonella foenum-graecum* L.):

Trigonella foenum-graecum L. commonly called Fenugreek or Methi (in Indian language) belongs to papilionoideae. Fenugreek, which has been produced for ages, is one of the most favorite fodder and medicinal plant of the Eastern Mediterranean and Subtropical climate countries. It is a widespread field crop. Due to its excellent adaptation abilities the plant species

can be produced as spring-sown plant in various countries of temperate climate. In Egypt, Morocco and India it is produced as a plant that survives the winter. When plant is green, it is mainly used to feed ruminants, however it is also suitable for producing green flours. Due to its high sugar content it can be ensilaged on its own, and as hay mixed with straw it is an invaluable fodder for horses improving the palatability of the fodder. The nutritional value of its seed is almost the same as that of soybean, however it has an additional advantage, not containing anti-nutritive components, therefore requiring no heat treatment (Sauvaire et al., 1976).

Fenugreek leaves are an important spice from Central Asia to Northern India; in Northern India, they are sometimes found in the typical yeast bread *naan* (then called *methi naan*); in South India, dried fenugreek leaves are often used as flavouring for potato curries and vegetables. The seed of fenugreek contains alkaloid, colin, bitter material, fatty acid, protein and vitamin C. The dominant aroma component in fenugreek seeds is a hemiterpenoid γ-lactone, sotolone (3-hydroxy 4,5-dimethyl 2(5H)-furanone). Plant products are rich in phosphorus, organic iron and carbohydrates. Its leaves are used both internally and externally to reduce swelling, prevent falling of hair and in the treatment of burns. Its seeds are carminative, antipyretic, anthelmintic and diuretic and are also useful in dropsy, chronic cough, heart diseases, bronchitis, piles and small pox. This plant is also found to possess antidiabetic property. The seeds contain diastase, alkaloids, and materials, which are excellent remedies for dysiorexia and weakness resulting from emaciation. It improves the appetite, it is a general health-restorer and it has a fattening effect, increases the number of red blood cells and restores the strength of a physically strained body.

C. Mothbean (*Vigna aconitifolia* L.):

The genus *Vigna* (Fabaceae, formerly Leguminosae) is composed of more than 200 species that are native to the warm regions of both the old world and new world. The genus *Vigna* contains several species that are of considerable economic importance in many developing countries. Cowpeas [*V. unguiculata* (L.) Walp.], mung beans [*V. radiata* (L.) Wilczek], and urid beans [*V. mungo* (L.) Hepper] are key dietary staples for many millions of people. *Vigna* species exhibit a

number of attributes that make them particularly valuable for inclusion in many types of cropping systems. They can be grown successfully in extreme environments (e.g., high temperatures, low rain fall, and poor soils) with few economic inputs. *Vigna* food products exhibit many excellent nutritional attributes. These provide a needed complement in diets comprised mainly of roots, tubers, or cereals. All parts such as tender green shoots and leaves, immature pods, and green and dry seeds, provide inexpensive protein available in several edible forms. These species are also valued as fodder, cover, and green manure crops. Two of the species, the moth bean and the mung bean, are of economic importance in the India.

Vigna aconitifolia (Jacq.) Marechal, commonly known as the Moth bean, Mat bean, Turkish gram, is native to India, Pakistan, and Burma (Rachie, 1974). The name "moth" comes from the Hindi word pronounced "mat" or "mote" according to the National Academy of Sciences. The moth bean is extensively grown for food in the arid and semi-arid regions of India. It is also an important pulse crop in semi-arid regions adjoining tropical dry regions. Moth bean is an annual food legume belonging to the subgenus *Ceratotropis* in the genus *Vigna*. Germination of moth bean is epigeal and spherical nitrifying nodules cover the root system. Moth bean is a hot weather, drought resistant legume.

2. OBJECTIVES

The objectives of this research were as under:

1. To isolate endophytic root-nodule bacteria from cultivated legumes and their phenotypic characterization
2. To assess plant growth-promotion (PGP) activities of root-nodule bacteria
3. To identify root-nodule bacteria employing partial 16S rDNA sequencing approach

Sub-objectives of the proposed research plan include

1. Field survey and collection of plant materials/nodules from selected sites.
2. Isolation and purification of endophytic root-nodule bacteria.
3. Evaluation of N_2 fixing ability.

4. Physiological and Biochemical characterization of endophytes associated with plant species selected for present study (as per methods described by Vincent, 1970).
5. Characterization of genetic diversity/polymorphism of isolates include
   (i) PCR amplification and polymorphism detection
   (ii) Sequencing of 16S rRNA and/or 16S-23S rDNA intergenic spacers (ITS region)
6. Phylogenetic or taxonomic analysis of the selected isolates using online bioinformatics tools.
7. Screening of isolates for Plant Growth Promoting (PGP) activities.

3. METHODOLOGY TO BE USED

Detailed work plan-

1. **Survey for nodule hunting:** Different field sites will be visited or surveyed in arid and semi-arid regions of Indian Thar Desert for nodule hunting. Isolates (root nodules) will be collected from plants growing at different geographical sites of arid and semi-arid regions.

2. **Isolation of rhizobacterial isolates under aseptic conditions from excavated nodules from natural sites:** Rhizobial isolates will be isolated from sterilized root nodules on different/specific/selective culture media.

3. **Analysis of physical conditions of soil of area under study:** The physical parameters of soil of area (from where nodules will be collected) will also be tested. These physical parameters include water holding capacity (WHC), humidity/moisture content, pH, salinity, alkalinity, electrical conductivity, nitrates, phosphorus, carbonates and bicarbonate estimation etc.

4. **Studies on Symbiotic traits, ability to fix nitrogen and Growth characteristics:** It includes the assessment of effectiveness in fixing atmospheric nitrogen and nodulation by different isolates under *in vitro* and *ex vitro* conditions. Growth characteristic include calculation of generation time.

5. **Physiological/Functional and Biochemical characterization:** This includes studies on
   1. Pigmentation, Colony morphology, Gram reactions, Cell Shape and Growth on N free medium
   2. Salt ($NaCl/CaCl_2$), Osmotic (PEG), Temperature stress and pH tolerance

3. Intrinsic antibiotic resistance profiling (IARP)
4. Heavy metal resistance
5. Whole cell SDS-PAGE protein profiling and gel analysis
6. Determination of ability to utilize different carbon source.
7. Determination of cellulase activity or lipo-polysaccharide (LPS) profiling.
8. Ability to hydrolyze the different polysaccharide.
9. Biotin prototrophy and Catalase reactions

6. **Genotypic characterization:** It includes-
   1. Extraction and purification of genomic DNA from isolates.
   2. PCR amplification of the DNA regions coding for the 16S rRNA genes, 16S-23S rDNA intergenic spacers (IGS/ITS), *nifH* or *nod* genes.
   3. RAPD fingerprinting, ARDRA analysis.
   4. Sequencing of the 16S rDNA/rRNA and/or 16S–23S ITS.
   5. Sequence alignment and phylogenetic/taxonomic analysis using bio-informatics tools.

7. **Screening of Isolates for plant growth promoting activity:** Screening of PGP activities includes:
   1. Determination of Phosphate solubilization, siderophore production, IAA like substances (Phytohormones)
   2. Assay for IAA production, ACC deaminase activity, Ammonia (NH_3) production and HCN Production
   3. Anti-fungal and anti-bacterial assay

4. REVIEW OF LITERATURE (National & International Status, Importance, patents etc.)

Legumes are simultaneously one of the largest families of crop plants occupying nearly all terrestrial biomes. The unusual flower structure, podded fruits and ability of the 88.0% species to form root nodules with compatible rhizobacteria define the legumes (Graham and Vance, 2003). The wide use of legumes as food crops, forages and green manures is mainly associated with

their ability to establish symbiotic associations with stem and root nodulating nitrogen (N_2) fixing bacteria, which are collectively referred as rhizobia. Rhizobia are of particular interest due to their symbiotic association with members of Leguminosae (Saleena *et al.*, 2001), which is the second largest family of flowering plants. These bacteria are among the most intensely studied groups of microorganisms mainly due to their potential to replace chemical N-fertilizers, with emphasis on their key role in achieving sustainability of tropical N-poor soils. These rhizobia are mostly soil bacteria and promote plant growth therefore referred as plant growth promoting rhizobacteria (PGPR) . PGPR are a heterogeneous group of bacteria that occur in rhizosphere, at root surfaces or in association with roots. PGPR improve the extent or quality of plant growth directly and /or indirectly. The direct promotion by PGPR entails either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of one or more phytopathogenic microorganisms. The exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indoleacetic acid, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick, 1995), (ii) symbiotic N_2 fixation, nodulation or nodule occupancy (Fuhrmann and Wollum, 1989) or asymbiotic N_2 fixation (Boddey and Dobereiner, 1995), (iii) solubilization of mineral phosphates and other nutrients (De Freitas *et al.*, 1997; Gaur, 1990) and (iv) antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan *et al.*, 1992) and cyanide (Flaishman *et al.*, 1996). However, role of cyanide production is contradictory as it may be associated with deleterious as well as beneficial rhizobacteria (Bakker and Schippers, 1987; Alstrom and Burns, 1989). In addition to these traits, plant growth promoting bacterial strains must be rhizospheric competent, i.e. able to survive in and colonize the rhizospheric soil (Cattelan *et al.*, 1999). Unfortunately, the interaction between associative PGPR and plants can be unstable and the good results obtained *in vitro* cannot always be reproduced under field

conditions (Chanway and Holl, 1993; Zhender *et al.*, 1999). The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effect on the plant-microbe interaction.

In last few decades a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance plant growth (Kloepper *et al.*, 1989; Okon and Labandera-Gonzalez, 1994; Glick, 1995). Recent information indicates that about 3000 bacterial taxa are capable of nodulating 400 taxa, while information is lacking for more than 40% of the genera (http://www.ildis.org). Until the early 1980s, all bacteria isolated from root nodules were classified into the genus *Rhizobium*, and speciation was based on nodulation with certain host plants, establishing the ''cross-inoculation group'' concept (Fred *et al.*, 1932). After that, numerical taxonomy considering several properties led to the definition of a new genus, *Bradyrhizobium*, and the renaming of some other species (Jordan *et al.*, 1984). Particularly in the last decade, ribosomal sequences with emphasis on the 16S rDNA have become the basis of bacterial molecular phylogeny and taxonomy (Garrity *et al.*, 2001) leading to the description of new rhizobial genera and species. Currently, rhizobia are positioned in four branches, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium-Sinorhizobium-Allorhizobium*, and non-symbiotic relatives within those branches may indicate common ancestry for rhizobial species and other parasitic or soil-borne bacteria (Garrity *et al.*, 2001; Wang *et al.*, 2000). However, in comparison to the number of nodulating-legume species, very few rhizobial species have been described. Genetic diversity of bacteria is being analyzed increasingly by PCR-based genomic fingerprinting methods. As more knowledge is acquired and isolates from unexplored legumes are studied, new species are discovered and former species are split. Due to improved methods of characterization, the classification of rhizobia has undergone drastic changes and the phylogenetic analysis of the family Rhizobiaceae and related genera has been upgraded (Chen *et al.*, 1988; Willems *et al.*, 1993; Yanagi *et al.*, 1993; de Lajudie *et al.*, 1994; Martínez-Romero *et al.*, 1996; Young *et al.*, 2001). Laguerre *et al.* (1994) applied the restriction fragment length

polymorphism (RFLP) analysis of PCR amplified 16S rRNA gene for identification of rhizobia. Since then, various workers have utilized this technique extensively and several novel species have been reported during the last decade (Amarger *et al.*, 1997; Wang *et al.*, 1998; Chen *et al.*, 2001; Tan *et al.*, 2001; Squartini *et al.*, 2002; Wang *et al.*, 2002). Applying these techniques, *Allorhizobium undicola* and all species of *Agrobacterium* have been reassigned to the genus *Rhizobium* (Young *et al.*, 2001). Pandey *et al.*, (2005) characterized PGPR isolates as *Burkholderia* from root nodules of *Mimosa* species using 16S rDNA gene analysis. Based on 16S rRNA gene sequencing Menna *et al.*, (2006) estimated molecular phylogeny of elite rhizobial strains used as commercial inoculants in Brazilian. Villegas *et al.*, (2006) identified nitrogen fixing *Sinorhizobium meliloti* from *Medicago laciniata* on the basis of PCR-RFLP analyses of 16S rDNA and the intergenic spacer (IGS) sequence between 16S and 23S rDNA regions. Similarly based on the sequencing of the 16S rRNA gene, *Bacillus thuringiensis* KR-1, *Enterobacter asburiae* KR-3, and *Serratia marcescens* KR-4 were characterized as non-rhizobial PGPR isolates from Nodules of Kudzu (*Pueraria thunbergiana*) (Selvakumar *et al.*, 2008). Jha *et al.* (2008) partially identified diazotrophic PGPR isolates from traditional Indian rice cultivars through amplification of *nifH* gene and sequencing of 16S rDNA gene. Binde *et al.* (2009) used rep-PCR fingerprinting and sequencing of 16s rDNA for taxonomic identification of 54 elite commercial rhizobial strains used as rhizobial inoculants in Brazil. Recently Taghavi *et al.*, (2009) used restriction analysis and sequencing of amplified 16S rRNA gene for identification and characterization of the endophytic bacteria exhibiting beneficial effects on growth and development of Poplar trees (a non-legume plant). Randomly amplified polymorphic DNA (RAPD) profiles have provided new tools for investigating genetic polymorphism. This method has been used for genetic analysis of *Bradyrhizobium* strains nodulating *Arachis hypogea (*van Rossum *et al.*, 1995) and nodule isolates of *Acacia* sp. (Khbaya *et al.*, 1998). Repetitive sequence-based PCR (rep-PCR) has come as an advanced technique, which is found to be in agreement with the phylogenies derived from multilocus enzyme analysis (de Bruijn *et al.*, 1992; Versalovic *et al.*, 1994). Pandey *et al.* (2004) applied rep-PCR fingerprinting along with 16S r

ARDRA and amplification of *nifH* gene for identification of genetic diversity of rhizobia from medicinal legumes growing in sub-Himalayan region of Uttaranchal (India). Solano *et al.*, (2006) applied PCR-RAPD analysis and 16S rDNA sequencing for screening and identification of isolates to improve the growth of *Cistus ladanifer* seedlings for reforestation of degraded Mediterranean ecosystems. Santillana *et al.,* (2008) characterized diversity of rhizobial isolates exhibiting different RAPD profiles from *Vicia faba* and *Pisum sativum* in Peru based on *rrs, atpD, recA* genes and 16S–23S intergenic sequence (IGS) analysis.

In India (particularly areas with nutritionally poor soil i.e. arid regions), economic and agronomic benefits can be achieved with legumes by research efforts focusing on N_2 fixation. Also the legumes, through their symbiotic abilities can play an important role in colonizing disturbed ecosystems. A number of laboratories are working with an objective of identifying the effective rhizobial strains for each legume species according to existing environmental conditions. The environmental factors include climate, weather conditions, soil characteristics and the composition or activity of the indigenous microbial flora of the soil. To achieve the maximum growth promoting interaction between PGPR and nursery seedlings or a crop plant, it is important to discover how the rhizobacteria exert their effects on plant and whether the effects are altered by various environmental factors, including the presence of other microorganisms (Bent *et al.*, 2001). The environmental constraints, particular plant species and bacterial strain and its compatibility with plant and environmental conditions influence the potential of rhizobacterial-legume symbiosis for nitrogen fixation and promotion of plant growth. Therefore, it is necessary to develop efficient strains in field conditions as per requirement of geographical and environmental conditions. Several cultivated leguminous crops find their habitats in hot arid regions. The microbial diversity associated with these plants can be used as resources for bioprospecting.

Beside cultivated crops, also there are number of native legumes in this region. In these legumes (legumes of stressed ecosystem i.e. Thar Desert), nitrogen fixation studies have been largely ignored as compared to agricultural and industrial crops of other regions. There are strong

possibilities that these plants may harbor those rhizobia or rhizobacteria, which can be important for increasing productivity in other cultivated legumes and for large-scale multiplication of other plants species. Selection of effective, efficient and compatible stress tolerant rhizobial strains could help in ecological rehabilitation of degraded soils and to increase soil fertility thereby improving the growth of associated plants of this region. One possible approach is to explore microbial diversity associated with these legumes for PGPR having combination of PGP activities and well adapted to particular environment. Searching for the most effective rhizobial strains is a time-consuming process involving the production of thousands of rhizobial cultures, and many greenhouse experiments and field trials. However, despite the considerable effort expended in selecting effective strains for almost hundreds legumes, their genetic characterization and taxonomic position is still poor. So keeping in view the above constrains, the present study was designed to isolate, screen and phylogenetically characterize the compatible rhizospheric bacterial isolates from commonly grown leguminous crops of arid and semi-arid regions for their multiple plant growth promoting activities. The commonly grown leguminous crops for present study will include chickpea, trigonella and mothbean. Also there are very less number of reports regarding studies on biological nitrogen fixation by these crop plants. The present study aims at gaining better understanding of phylogenetic relationships, based on the sequencing of the whole 16S rRNA, 16S–23S IGS from rhizobial isolates from arid and semi-arid regions of Indian Thar Desert/ Rajasthan.

5. RELAVANCE OF WORK TO SOCIETY

This work aims to isolate rhizobacteria nodulating different cultivated crops (namely chickpea, mothbean and fenugreek) from arid and semi-arid regions of Rajasthan and evaluate their biodiversity. Analysis of auxanographic and symbiotic characteristics, together with restriction fragment length polymorphism analysis of the PCR-amplified 16S rRNA genes, 16-23s ITS regions allowed us to identify effective and competitive strains tolerant to various climatic conditions and to show great diversity among these rhizobia. Therefore an effective cultivar-rhizobia strain combination can be selected/ defined for areas under study.

6. FUTURE PLAN

As there are many environmental stresses that adversely affect nodulation and potential of legume-rhizobium symbiotic relationship for nitrogen fixation. If the proposed project is implemented, an effective cultivar-strain combination resistant to different environmental stresses can be formulated for crop species selected for present investigation. Also the rhizobia are highly specific for host legumes. Broader host range would simplify production, distribution, and grower use of rhizobia. Future prospects include overcoming the specificity of PGPR strain or selection of PGPR strain with broader host range, so that these PGPR can benefit a wide range of crops. After identification of an effective strain or broad range strain, packaging and formulation of inoculants can be carried out to minimize adverse effects of environmental stresses. This could help in ecological rehabilitation of degraded soils and to increase soil fertility of hot arid or desert regions.

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